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Perspectives and Commentaries

Potential of Colony-forming Cell Assays in the Evaluation of Ovarian Cancer

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(A COMMENT ON: Runge HM, Neumann HA, Bauknecht T, Pfleiderer A. Growth patterns and hormonal sensitivity of primary tumor, abdominal metastasis and ascitic fluid from human epithelial ovarian carcinomas in the tumor colony-forming assay. *Eur J Cancer Clin Oncol* 1986, **22**, 691–696.)

CONSIDERABLE knowledge has accumulated in the past few years on the use of human tumor colony-forming cell assays in the evaluation of tumor biology.

Runge *et al.* have provided some interesting data about 12 patients whose ovarian cancer samples grew more than 30 colonies per dish in a double-layer soft agar assay. They have observed that the metastatic site had a higher cloning efficiency than the primary tumor. This observation has been reported by others, cited by the authors, and is one biological feature of colony-forming cell assays. These assays detect anchorage-independent growth of plated cells, a property that seems determinant for tumor growth. One may criticize the fact that no attempt was made by any of these authors to correct their plating efficiencies for the proportion of tumor cells present in their sample [1]. However, the different cloning efficiencies are certainly correlated with some biological characteristics of tumor cells. For example, in several tumor types, high cloning efficiencies of the patient's samples have been prognostic of a short survival [2].

As the authors point out, there may well be some difference in chemosensitivity patterns of primary and metastatic cells. This is an open question; but even if the answer is yes, the predictive value of these assays may be adequate in some situations. Patients with metastatic ovarian cancer, refractory to primary treatment, will often have cells available for chemosensitivity testing. These patients have little to expect from second-line chemotherapy [3]. Preliminary data indicate that such patients would benefit more from a test-directed treatment than from blind treatment [4]. Twenty-four patients relapsing from standard combination chemotherapy have been treated according to the results of a human tumor clonogenic assay. They had a 55% objective response rate and a 7 month median survival, with 25% surviving after 21 months. Some who had 'sensitive' tumors (28 patients), and those who had 'resistant' tumors (31 patients) were treated empirically. These 59 patients had the same 15–18% response rate and 3–4 months median survival.

Runge *et al.* had a very low number of samples that grew sufficiently in their assay system. Many reasons can explain why some groups are more successful than others in obtaining growth of ovarian cancer cells. This technical problem makes it difficult to propose widespread use of the assays for individualized testing of drugs. One of the aims of the EORTC Clonogenic Assay Screening Study Group is to provide a basis for improvement of assay conditions and quality control in individual laboratories. Progress is encouraging and study 37861 (unpublished) has shown a high degree of inter-laboratory reproducibility.

As individualized predictive testing in ovarian cancer remains difficult for many groups, another

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promising area of investigation would be to evaluate new drugs in this system, before clinical phase II trials. It might be possible to predict the patterns of sensitivity to several drugs before their clinical use. The EORTC Clonogenic Assay Screening Study Group conducted a confirmatory assay looking at cisplatin, carboplatin and iproplatin *in vitro*. Sixty-one ovarian cancer samples were acquired within 5 months, and drug testing showed a remarkable parallelism for sensitivity and resistance between the three analogs (study 37842, unpublished). Similar approaches have been conducted by many individual laboratories or cooperative groups. If due attention is paid to several pitfalls (e.g. drug concentrations that are irrelevant), we should see widespread application of many techniques for '*in vitro* phase II' studies.

The other major aspect of the article we review is the evaluation of hormonal sensitivity of ovarian cancer and their metastases. The authors used relatively high doses of tamoxifen (10^{-6} M) in order to observe a 30% or less colony survival of treated samples. This is about three to five times more than the serum concentration obtained in women treated with tamoxifen 10 mg/m^2 twice daily [5]. We can thus conclude that the assay, as used by the authors, could have predicted the lack of meaningful clinical usefulness of tamoxifen [3]. The limited number of observations does not allow any conclusion about the possible correlation between estrogen-receptor (ER) positivity and *in vitro* response to tamoxifen. Two out of three ER negative samples are among the most resistant ones.

Colony forming cell assays thus have several uses. A few have been already mentioned (phase II *in vitro* studies of hormones and chemotherapy; prognostic value of *in vitro* growth). We would like to mention just a couple, in therapeutic or prognostic areas related to ovarian cancer. The assays are useful for understanding some mechanisms of action of biological response modifiers. For example, it has been shown that adherent cells from ascites became strongly inhibitory to tumor colony growth after exposure to recombinant interferon-gamma [6]. Such observations should encourage non-cytotoxic trials of these agents. Tumor necrosis factor (TNF) is another agent that has been evaluated with a colony-forming cell assay. In contrast to breast cancer cells, ovarian cancer cells are quite resistant to *in vitro* treatment with TNF [7]. It remains to be seen whether modulation of accessory cells could overcome this resistance or if high intraperitoneal concentrations of TNF can be achieved. As a last example we mention the possible use of such assays to distinguish aneuploid cells with different growth characteristics and thus refine the prognostic value of ploidy [8].

In spite of several restrictions, we feel that the 'credibility gap' of human tumor colony-forming cell assays has been bridged. As with any other biological test, careful development and avoidance of excessive hopes will allow us to realize the possible usefulness of similar assays.

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